54. (New) The method of Claim 1, wherein the second mutant form of the reporter enzyme is linked to the C-terminal of the arrestin.

55. (New) The method of Claim 14, wherein the modified GPCR fusion protein comprises a full length GPCR linked to the first mutant form of reporter enzyme by a first polypeptide linker comprising one or more serine/threonine clusters.

56. (New) The method of Claim 14, wherein the protein partner is an arrestin.

B2 57. (New) The method of Claim 14, wherein the modified GPCR and the first mutant form of reporter enzyme are linked together by a polypeptide linker represented by the formula -$(GGGGS)_n$-.

58. (New) The method of Claim 57, wherein n is 2 or more.

59. (New) The method of Claim 57, wherein n is 4.

60. (New) The method of Claim 56, wherein the second mutant form of the reporter enzyme is linked to the C-terminal of the arrestin. --

REMARKS

Support for newly added Claims 26-60 can be found in the specification at the following locations: page 20, lines 12 and 20; page 21, lines 7 and 15; page 23, lines 18-21; page 24, lines 2 and 11; page 31, line 18 - page 34, line 10; and in FIGS. 10A-10P, 11A-11L, 12A-12L, 13A-13L, 14-22, 24 and 25.

Claims 6-8 were rejected under 35 U.S.C. §112, first paragraph. This rejection is respectfully traversed. Claims 6-8 are directed to a DNA molecule comprising a sequence encoding a biologically active hybrid arrestin, a DNA construct comprising the DNA molecule, and a cell transformed with the DNA construct, respectively. It is respectfully submitted that the specification provides an enabling disclosure for each of Claims 6-8. In particular, the DNA

molecule as set forth in Claim 6 can be used in a DNA construct (e.g., the DNA construct of Claim 7) to direct the expression of a hybrid GPCR in a cell (e.g., as described in Claim 8). The cell as set forth in Claim 8 can be used, for example, in a GPCR assay. That is, the cell as set forth in Claim 8 can be contacted with a DNA construct capable of directing the expression of a fusion protein of a GPCR and a second, complementary mutant form of the reporter enzyme (See, for example, Claim 4) and the resulting cell can be used in an assay (e.g., in an assay to determine ligands for orphan GPCRs as described in FIG. 28). Therefore, it is respectfully submitted that the application, when filed, contained information regarding the subject matter of Claims 6-8 which, when coupled with information known in the art, would enable one skilled in the art to make and use the invention as defined by these claims without undue experimentation. See MPEP §2164.01. Accordingly, it is respectfully requested that the rejection of Claims 6-8 be reconsidered and withdrawn.

Claims 1, 6-10, 13, 15, 18, 24 and 25 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Blau (U.S. Patent No. 6,342,345 B1) in view of Barak (U.S. Patent No. 5,891,646) and further in view of Kovoor, Gurevich '95 and Gurevich '97. This rejection is respectfully traversed for the reasons set forth below.

First, it is respectfully submitted that there is no teaching or suggestion in either Barak or Blau to combine the references in the manner suggested in the Official Action. In particular, Blau broadly discloses methods and compositions for detecting protein-protein interactions using fusion proteins of β-galactosidase mutants. However, G-protein coupled receptors are not specifically disclosed by Blau. Further, while Barak teaches an assay method for GPCRs, the assay method of Barak does not involve the complementation of mutant forms of reporter enzyme (i.e., neither the GPCR nor the β-arrestin molecule in Barak are expressed as a fusion protein to a

mutant form of reporter enzyme). In order to arrive at the Applicant's invention from the teachings of Barak and Blau, the Official Action makes the following unsupported assertions:

> An artisan of ordinary skill in the art of molecular biology would have recognized that the method of Barak, et al. was limited by the fact that it did not allow detection of the direct interaction of the fluorescent labeled β-arrestin employed therein with a specific G protein-coupled receptor. (page 7 of the Official Action mailed June 10, 2002)
>
> That artisan would have realized that the fluorescent labeled β-arrestin would have accumulated at the cell membrane in response to the activation of any G protein-coupled receptor which might be present in the cell. (page 7 of the Official Action mailed June 10, 2002)
>
> . . . that artisan would have appreciated the fact that an accurate measurement of the ligand activation of a particular receptor by employing the method of Barak, et al. would require the inclusion of a control consisting of a cell which is otherwise identical to the test cell except for the absence of the receptor of interest. (page 7 of the Official Action mailed June 10, 2002)
>
> That artisan would have understood that the method of detecting protein-protein interaction that was described by Blau, et al. would not have required such a control because it measured the direct interaction of two specific proteins and, therefore, would allow one to measure the direct interaction of β-arrestin with a specific G protein-coupled receptor in an intact cell irrespective of the interaction of β-arrestin with any other G protein coupled receptor which might be present in that cell. (page 7 of the Official Action mailed June 10, 2002)
>
> Therefore, that artisan would have found it *prima facie* obvious to have employed the β-galactosidase complementation system of Blau, et al. to detect the interaction of β-arrestin with a particular G protein-coupled receptor to identify agonists and antagonists thereto as taught by Barak, et al. because that artisan would have been more confident that the results obtained by the method of Blau, et al. were representative of the action of the particular receptor of interest. (pages 7-8 of the Official Action mailed June 10, 2002)

It is respectfully submitted that the above line of reasoning could only have been arrived at with the benefit of the Applicant's disclosure. In particular, the Official Action makes reference to what one of ordinary skill in the art would have "recognized", "realized", "appreciated", "understood" or "found" without providing support for these statements. It is respectfully submitted that the alleged shortcomings of the Barak assay method referred to in the

Official Action are only apparent given the Applicant's disclosure of an improved receptor function assay for G-protein coupled receptors.

As set forth in The MPEP:

> . . . the examiner must step backward in time and into the shoes worn by the hypothetical "person of ordinary skill in the art" when the invention was unknown and just before it was made. In view of all factual information, the examiner must then make a determination whether the claimed invention "as a whole" would have been obvious at that time to that person. Knowledge of Applicant's disclosure must be put aside in reaching this determination . . . The tendency to resort to "hindsight" based upon Applicant's disclosure is often difficult to avoid due to the very nature of the examination process. However, impermissible hindsight must be avoided and the legal conclusion must be reached on the basis of the facts gleaned from the prior art. MPEP §2142.

In view of the above, it is respectfully submitted that the rejection is an impermissible hindsight reconstruction of the Applicant's invention. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

Additionally, it is respectfully submitted that the modification of the Barak reference in the manner set forth in the Official Action would fail to establish a case of *prima facie* obviousness since the modification would involve a change in the principle of operation of the reference. It is well established that if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. In re Ratti, 270 F.2d 810, 123 USPQ 349 (CCPA 1959). In Ratti, the court reversed an obviousness rejection holding that the "suggested combination of references would require a substantial reconstruction and redesign of the elements shown in [the primary reference] as well as a change in the basic principle under which the [primary reference] construction was designed to operate." 270 F.2d at 813, 123 USPQ at 352.). See MPEP 2143.01.

Similarly, the modification proposed in the Official Action would require a substantial reconstruction and redesign of the assay method of Barak. Namely, in the assay methods of Barak, the test cell expresses GPCR and a conjugate of β-Arrestin and a visually detectable molecule. According to Barak, the test cell is then observed for evidence of translocation of the detectable molecule (see, for example, column 2, lines 35-37 of Barak). For example, the translocation of the visually detectable molecule (e.g., from the cytosol to the cell edge) can be used to assay G-protein coupled receptor activity (see, for example, column 2, lines 43-52 of Barak). Thus, in Barak the movement of a visually detectable molecule bound to the β-arrestin molecule is being monitored. The principle of operation of Barak is therefore substantially different than the principle of operation of the invention as defined by Claim 1 wherein *the complementation of mutant forms of a reporter enzyme*, one expressed as a fusion protein to a GPCR and another as a fusion protein to an interacting protein partner, is being monitored. Accordingly, it is respectfully submitted that the invention as set forth in Claims 1 and 6-9 is patentable over the cited references. Therefore, in view of the above, reconsideration and withdrawal of the rejection of Claims 1 and 6-9 is respectfully requested.

Claims 15, 18 and 25 depend from Claim 1. Claims 10, 13 and 24 depend either directly or indirectly from Claim 9. These claims are therefore also patentable over the cited references for at least the reasons set forth above with respect to Claims 1 and 9, respectively. Reconsideration and withdrawal of the rejections of these claims is therefore also respectfully requested.

Claims 26-60 have been added. These claims depend either directly or indirectly from Claims 1-9 or 14 and are therefore also patentable over the references of record for at least the reasons set forth above. Additionally, Claims 26-30, 32-55 and 57-60 can be further distinguished from the references of record. In particular, each of Claims 26-28, 32-34, 37-39,

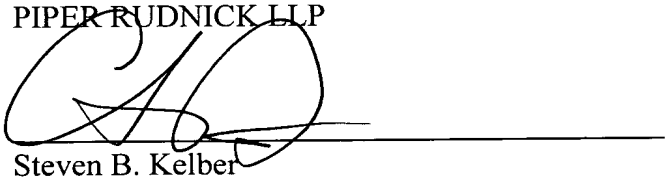
41-43, 45-47, 51-53 and 57-59 either recite or depend from a claims which recites that the GPCR and the first mutant form of reporter enzyme are linked together by a polypeptide linker represented by the formula -$(GGGGS)_n$-. Further, Claims 29, 35, 48-50, 54 and 60 each recite that the second mutant form of the reporter enzyme is linked to the C-terminal of the arrestin. Additionally, Claims 30, 36, 40, 44 and 55 each recite that the modified GPCR fusion protein comprises a full length GPCR linked to a mutant form of reporter enzyme by a first polypeptide linker comprising one or more serin/threonine clusters. There is no teaching or suggestion in the references of record of a method, a DNA molecule or a DNA construct as set forth in these claims. Accordingly, it is respectfully submitted that Claims 26-60 are patentable over the references of record.

CONCLUSION

All rejections having been addressed by the present amendments and response, Applicants believe that the present case is in condition for allowance and respectfully request early notice to that effect. If any issues remain to be addressed in this matter which might be resolved by discussion, the Examiner is respectfully requested to call Applicants' undersigned counsel at the number indicated below.

Respectfully submitted,

PIPER RUDNICK LLP



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MARKED-UP COPY OF AMENDED CLAIMS

1. (Twice Amended) A method of assessing the effect of a test condition on G-protein-coupled receptor (GPCR) pathway activity, comprising:

a) providing a cell that expresses a GPCR as a fusion protein to a first mutant form of a reporter enzyme and an arrestin as a fusion protein to a second mutant form of the reporter enzyme complementary to the first mutant form of the reporter enzyme,

wherein [said] the arrestin is modified to enhance binding of said arrestin to [said] the GPCR, wherein said enhanced binding between said arrestin and [said] the GPCR increases sensitivity of detection of said effect of [said] the test condition;

b) exposing the cell to a ligand for said GPCR under [said] the test condition; and

c) monitoring activation of said GPCR by complementation of [said] the first and second mutant forms of the reporter enzyme;

wherein increased reporter enzyme activity in the cell compared to that which occurs in the absence of [said] the test condition indicates increased GPCR interaction with the modified arrestin compared to that which occurs in the absence of [said] the test condition, and decreased reporter enzyme activity in the cell compared to that which occurs in the absence of [said] the test condition indicates decreased GPCR interaction with the modified arrestin compared to that which occurs in the absence of [said] the test condition.

2. (Amended) A method of assessing the effect of a test condition on G-protein-coupled receptor (GPCR) pathway activity, comprising:

a) providing a cell that expresses a GPCR as a fusion protein to [one] a first mutant form of reporter enzyme and an interacting protein partner as a fusion to [another] a second mutant form of the reporter enzyme;

wherein [said] the GPCR fusion protein is modified to include one or more sets of serine/threonine clusters, wherein said one or more sets of serine/threonine clusters enhance binding of said GPCR to arrestin, wherein said enhanced binding between said GPCR and said arrestin increases sensitivity of detection of said effect of said test condition;

b) exposing the cell to a ligand for [said] the GPCR under said test condition; and

c) monitoring activation of [said] the GPCR by complementation of said reporter enzyme;

wherein increased reporter enzyme activity in the cell compared to that which occurs in the absence of [said] the test condition indicates increased GPCR interaction with [said] the interacting protein partner compared to that which occurs in the absence of [said] the test condition, and decreased reporter enzyme activity in the cell compared to that which occurs in the absence of [said] the test condition indicates decreased GPCR interaction with interacting protein partner compared to that which occurs in the absence of [said] the test condition.

3. (Amended) A DNA molecule comprising a sequence encoding a biologically active hybrid GPCR, wherein [said] the hybrid GPCR comprises a GPCR as a fusion protein to [one] a mutant form of reporter enzyme and wherein [said] the hybrid GPCR is modified to include one or more sets of serine/threonine clusters, wherein [said] the one or more sets of serine/threonine clusters enhance binding of said hybrid GPCR to an arrestin.

4. (Amended) A DNA construct capable of directing the expression of a biologically active hybrid GPCR in a cell, comprising the following operatively linked elements:

a promoter; and

a DNA molecule comprising a sequence encoding a biologically active hybrid GPCR, wherein [said] the hybrid GPCR comprises a GPCR as a fusion protein to one mutant form of reporter enzyme and wherein [said] the hybrid GPCR is modified to include one or more sets of serine/threonine clusters, wherein [said] the one or more sets of serine/threonine clusters enhance binding of [said] the hybrid GPCR to an arrestin.

5. (Amended) A cell transformed with a DNA construct capable of expressing a biologically active hybrid GPCR in a cell, comprising the following operatively linked elements:

a promoter; and

a DNA molecule comprising a sequence encoding a biologically active hybrid GPCR, wherein [said] the hybrid GPCR comprises a GPCR as a fusion protein to one mutant form of reporter enzyme and wherein [said] the hybrid GPCR is modified to include one or more sets of serine/threonine clusters, wherein [said] the one or more sets of serine/threonine clusters enhance binding of [said] the hybrid GPCR to an arrestin.

6. (Twice Amended) A DNA molecule comprising a sequence encoding an arrestin as a fusion protein to a mutant form of a reporter enzyme, wherein [said] the arrestin is modified to enhance binding of [said] the arrestin to a GPCR.

7. (Twice Amended) A DNA construct comprising the following operatively linked elements:

a promoter; and

a DNA molecule comprising a sequence encoding an arrestin as a fusion protein to a mutant form of a reporter enzyme, wherein [said] the arrestin is modified to enhance binding of said arrestin to a GPCR.

8. (Twice Amended) A cell transformed with a DNA construct comprising the following operatively linked elements:

a promoter; and

a DNA molecule comprising a sequence encoding an arrestin as a fusion protein to a mutant form of a reporter enzyme, wherein [said] the arrestin is modified to enhance binding of [said] the arrestin to a GPCR.

9. (Twice Amended) A method of assessing the effect of a test condition on G-protein-coupled receptor (GPCR) pathway activity, comprising:

a) providing a cell that expresses a GPCR as a fusion protein to a first mutant form of a reporter enzyme and an arrestin as a fusion protein to a second mutant form of the reporter enzyme complementary to the first mutant form of the reporter enzyme,

wherein [said] the arrestin is modified by introducing a point mutation in a phosphorylation-recognition domain to remove a requirement for phosphorylation of [said] the GPCR for arrestin binding to permit binding of [said] the arrestin to said GPCR in [said] the cell regardless of whether [said] the GPCR is phosphorylated,

b) exposing the cell to a ligand for said GPCR under [said] the test condition; and

c) monitoring activation of [said] the GPCR by complementation of [said] the first and second mutant forms of the reporter enzyme;

wherein increased reporter enzyme activity in the cell compared to that which occurs in the absence of [said] the test condition indicates increased GPCR interaction with the modified arrestin compared to that which occurs in the absence of [said] the test condition, and decreased reporter enzyme activity in the cell compared to that which occurs in the absence of [said] the test condition indicates decreased GPCR interaction with the modified arrestin compared to that which occurs in the absence of [said] the test condition.

10. (Amended) The method of Claim 9, wherein [said] the arrestin is mutated to increase a property selected from affinity and avidity for activated, non-phosphorylated GPCR.

11. (Amended) The method of Claim 10, wherein [said] the arrestin is β-arrestin2 and wherein [said] the β-arrestin2 is mutated to convert Arg169 to an oppositely charged residue.

12. (Amended) The method of Claim 11, wherein [said] the oppositely charged residue is selected from the group consisting of histidine, tyrosine, phenylalanine and threonine.

13. (Amended) The method of Claim 9, wherein [said] the arrestin is mutated to increase a property selected from affinity and avidity for activated and phosphorylated GPCR.

14. (Amended) A method of assessing the effect of a test condition on G-protein-coupled receptor (GPCR) pathway activity, comprising:

a) providing a cell that expresses a GPCR as a fusion protein to [one] a first mutant form of a reporter enzyme and an interacting protein partner as a fusion to [another] a second mutant form of the reporter enzyme;

wherein [said] the GPCR fusion protein is modified to include one or more [sets of] serine/threonine clusters, [said] the one or more serine/threonine clusters defined as serine or threonine residues occupying three consecutive or three out of four consecutive positions [in a carboxyl-termini of said GPCR], wherein said one or more [sets of] serine/threonine clusters enhance binding of [said] the GPCR to arrestin, wherein [said] the enhanced binding between [said] the GPCR and [said] the arrestin increases sensitivity of detection of [said] the effect of [said] the test condition;

b) exposing the cell to a ligand for [said] the GPCR under [said] the test condition; and

c) monitoring activation of [said] the GPCR by complementation of [said] the reporter enzyme;

wherein increased reporter enzyme activity in the cell compared to that which occurs in the absence of [said] the test condition indicates increased GPCR interaction with [said] the interacting protein partner compared to that which occurs in the absence of [said] the test

condition, and decreased reporter enzyme activity in the cell compared to that which occurs in the absence of [said] the test condition indicates decreased GPCR interaction with interacting protein partner compared to that which occurs in the absence of [said] the test condition.

15. (Amended) The method of Claim 1, wherein [said] the modified arrestin exhibits enhanced binding to activated, phosphorylated GPCR.

16. (Twice Amended) The method of Claim 1, wherein [said] the modified arrestin comprises conversion of Arg169 to an amino acid selected from the group consisting of histidine, tyrosine, phenylalanine and threonine.

17. (Twice Amended) The method of Claim 1, wherein [said] the modified arrestin comprises conversion of Val170 to alanine.

18. (Twice Amended) The method of Claim 1, wherein [said] the arrestin is selected from the group consisting of β-arrestin1 and β-arrestin2, and wherein [said] the β-arrestin1 or [said] the β-arrestin2 is truncated for all or part of a carboxyl-terminal half of [said] the β-arrestin1 or [said] the β-arrestin2.

19. (Amended) The method of Claim 18, wherein [said] the β-arrestin1 or [said] the β-arrestin2 is truncated from amino acid 190 of [said] the β-arrestin1 or [said] the β-arrestin2 to [said] the carboxyl-terminal end of [said] the β-arrestin1 or [said] the β-arrestin2.

20. (Twice Amended) The method of Claim 1, wherein [said] the arrestin is a chimera of β-arrestin1, β-arrestin2 and/or visual arrestin.

21. (Amended) The method of Claim 10, wherein [said] the arrestin is a chimera of β-arrestin1, β-arrestin2 and/or visual arrestin.

22. (Amended) The method of Claim 11, wherein [said] the arrestin is a chimera of β-arrestin1, β-arrestin2 and/or visual arrestin.

23. (Amended) The method of Claim 12, wherein [said] the arrestin is a chimera of β-arrestin1, β-arrestin2 and/or visual arrestin.

24. (Twice Amended) The method of Claim 10, wherein [said] the arrestin is β-arrestin2 and wherein [said] the β-arrestin2 is mutated to convert Arg170 to an oppositely charged residue.

25. (Twice Amended) The method of Claim 1, wherein [said] the modified arrestin comprises conversion of Arg170 to an amino acid selected from the group consisting of histidine, tyrosine, phenylalanine and threonine.